Remarks:

The claims have been represented in accordance with current rules of amendment practice because claim 22 was inadvertently omitted in the Appendix of the most recent Office Action response. Accordingly, all of the presently pending claims are merely restated. No other amendments have been made and no new matter has been added.

IV. RESPONSE TO REJECTIONS UNDER 35 U.S.C. § 103(a)

(i) The Law

"A claimed invention is unpatentable if the differences between it and the prior art "are such that the subject matter as a whole (emphasis added) would have been obvious at the time the invention was made to a person of ordinary skill in the art." *In re Dembiczak*, 175 F.3d 994, 998, 50 U.S.P.Q.2d 1614, 1616 (Fed. Cir. 1999). In determining whether a claimed invention is obvious one must consider; 1) the scope and content of the prior art; 2) the level of skill in the prior art; 3) the differences between the claimed invention and the prior art; and 4) objective evidence of non-obviousness such as secondary factors. *Id.*

The PTO bears the burden under 35 USC § 103 to establish an unrebutted *prima facia* case of obviousness. *In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453, ___ (Fed. Cir. 1998). To satisfy its burden, the PTO must show some objective teaching in the prior art or that knowledge generally available in the art would lead the ordinary practitioner to combine relevant teaching. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). In the absence of a proper *prima facia* case of obviousness, an Applicant is entitled to a patent. *In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453, ___ (Fed. Cir. 1998). To overcome a claimed *prima facia* case of obviousness, an Applicant can either show that the *prima facia* case of obviousness is insufficient because it relies on incorrect factual predicates or otherwise present secondary evidence of non-obviousness. *Id.*

A proper rejection under 35 USC § 103 may not be premised upon "bald assertions" for which there is no support for or explanation of a conclusion. *Id.* An Examiner's cursory statement unaccompanied by evidence or reasoning is entirely inadequate to support a rejection. *In re Sichert*, 566 F.2d 1154, 1164, 196 U.S.P.Q. 209, 217 (C.C.P.A., 1977). A rejection based on section 103 must be based in fact that is not aided by hindsight. *In re Warner*, 54 C.C.P.A. 1628, 1635, 379 F.2d 1011, 1017, 154 U.S.P.Q. 173, 178 (C.C.P.A., 1967). The PTO may not resort to speculation, unfounded assumptions or hindsight reconstruction to supply deficiencies in its factual basis for a rejection. *Id.* Doubts as the factual basis for a rejection must to resolved in favor of the Applicant since it is the PTO's burden to establish a *prima facia* case of obviousness. *Id.*

(ii) The Facts

- The Examiner acknowledges that Kosse does not teach enzyme-linked probes for the analysis of yeast, including *Dekkera bruxellensis* (OA at page 3).
- The Examiner has specifically stated that Kosse: "... teaches that prior to *in situ* hybridization, yeast cell walls **must be permeablized** (emphasis added) and that probes **must be selected to yeast 18 rRNA which are fully accessible to probes** (emphasis added) (see page 478)." (OA at page 3)
- Kosse specifically describes the importance of permeablization of the cell wall of yeast else probes will not penetrate and the yeast cannot be determined (Kosse at page 474, col. 1, first full paragraph). Kosse specifically describes treatment of the yeast cells with **lyticase** to permeablize the cells to the flourescently labeled probes. *Id.*
- Kosse specifically teaches that only the 3' end of the 18S rRNA is accessible to fluorescently labeled probes and that the other variable regions of 18S rRNA were not accessible to *in-situ* hybridization. (Abstract and page 478, col. 2, first full paragraph).
- The Examiner acknowledges that Kosse teaches *in-situ* assays using fluorescently labeled probes whilst teaching dot-blot assays using digoxigenin labeled probes (OA at page 3).
- Amann et al. (Reference CA) specifically teach that enzyme-linked probes WOULD NOT penetrate into yeast cells (Abstract and pages 3008-3010, section entitled "Penetration of HRP-labeled oligonucleotides into whole fixed cells). Amann et al. specifically explain that with a horseradish peroxidase label, the oligonucleotide probe is approximately 100 time larger than is a flourescently labeled probe (Amman et al. at page 3008, bottom of col. 2). Amann et al. also teach that cells of *Saccharomyces cerevisiae* (a yeast) were not determinable with enzyme-linked probes even when treated with enzymes (**lyticase** and β-glucoronidase) or detergents (Amann at page 3010, middle, col. 1). It is noted that **lyticase** is the same enzyme that Kosse used to permiabilize yeast to fluorescently labeled

oligonucleotide probes and Amann et al. specifically teach that said **lyticase** enzyme does not work to permiabilize *Saccharomyces cerevisiae* to enzyme-linked oligonucleotide probes. Stender (1998), discussed below, is silent to this particular issue.

- Stender (1998) does not teach anything about yeasts but is limited to determinations of mycobacteria.
- Stender (1998) teaches enzyme-linked probes for the determination of mycobacteria but does not teach about permiabilizing yeast cells to enzyme-linked probes.
- Stender (1998) did not actually use enzyme-linked probes in any assay and does not appear to have appreciated the difficulty that Amann et al. specifically describes with regard to getting large probes (e.g. enzyme-linked probes) into cells having a cell wall.

*(iii) **Rebuttal To The Rejection Based Upon Kosse And Stender (1998)***

At paragraph 3 of the present Office Action, the Examiner has rejected claims 1-8 and 46 under 35 U.S.C. §103(a) as being unpatentable over Kosse in view of Stender (1998). Applicants respectfully traverse this rejection and stand ready to appeal this rejection directly to the Board of Patent Appeals & Interferences if again maintained.

As stated above with regard to the law of 35 U.S.C. §103(a), it behooves The Office to properly state the basis for rejecting claimed subject matter else the Applicant is entitled to the grant of his letters patent. It is further submitted that a proper rejection under 35 U.S.C. §103(a) must recite not only where each of the elements/limitations of the claimed subject matter are found in two or more references but The Office must also establish a clear **motivation**, based upon the teachings of the references, to combine the references in the manner presently claimed. It is respectfully submitted that the rejection articulated at paragraph 3 of the present Office Action does not state any **specific motivation** to combine the references and is therefore *prima facia* deficient. Moreover, it is believed that the statement of the rejection is hindsight based and self-fulfilling. Accordingly, it is respectfully submitted that the stated rejection of claims 1-8 and 46 over Kosse in view of Stender (1998) should properly be withdrawn.

In addition to the foregoing, it is respectfully submitted that based upon the facts set forth above, the ordinary practitioner is without any proper motivation or reasonable expectation of successfully applying an enzyme-linked *in-situ* probe for the determination of yeast. Amann et al. is the most specific teaching on this subject matter and it **clearly teaches away** from the presently claimed subject matter.

At pages 4-5 of the Office Action the Examiner presents her rebuttal to Applicants previously submitted arguments. In particular the Examiner argues: "... **there are no teachings in Amann which indicate that the enzyme linked probes cannot be applied to the detection of yeasts** (emphasis added). Amann provides the results obtained when applying the enzyme-linked probes to the detection of S. cerevisiae. However, Amann does not teach that these results apply to the detection of all yeast. While Amann teaches that an enzyme-labeled probe was not useful for the detection of Saccharomyces cerevisiae, the reference teaches that modifying the conditions for permiabilization of cells allows one to use enzyme-labeled probes for some organisms." (OA at page 4)

What a reference teaches is a question of fact. *In re Bell*, 991 F.2d 781, 784, 26 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1993). The Examiner states her opinion about what she believes Amann et al. teach without making any reference to any text that might support her position. Most importantly however, the Examiner's arguments do not properly address the issue of whether or not the ordinary practitioner would have both motivation and a reasonable expectation of successfully getting an enzyme-linked probe into a yeast after reading Amann et al. or whether the ordinary practitioner would have been dissuaded from attempting such an assay. Applicants believe that the ordinary practitioner would have been dissuaded from performing such an assay and therefore most emphatically believe that Amann et al. **teach away** from the presently claimed subject matter.

Amann et al. make the following specific statements.

"Hitherto we had not achieved penetration of enzyme-labeled probe into gram positive bacteria or **yeast cells** (emphasis added)." Amann et al., Abstract, page 3007.

"However, since the high-molecular weight enzyme-antibody conjugate must penetrate the cell wall of the fixed target cells, this approach has not served to visualize any gram-positive bacteria so far examined." Amann et al. at page 3007, col. 2, lines 3-7.

"The molecular weight of horseradish peroxidase (40,000) is approximately 100 times greater than that of the fluorsecein or tetramethylrhodamine, the two most common labels of rRNA-targeted oligonucleotide probes for single-cell identification. This increases the overall molecular weight of the probe from about 6,000 to about 50,000, and penetration of enzyme-linked probe through the cell periphery might be expected to hinder whole-cell identification." Amann et al. at page 3008, col. 2, lines 51-59.

"On the other hand, the gram-positive bacteria examined (B. subtilis and several strains of lactococci) remained **impermeable to the HRP-labeled probe** even after prolonged incubation with lysozyme, lysostaphin (Sigma), or mutanolysin (Sigma) at different concentrations. Following extensive digestion, the gram-positive bacteria were only partly stained while gram positive bacteria, added as a control, were lysed (data not shown)." Amann et al. at page 3010, col. 1, lines 5-13.

"In an attempt to increase hybridization of the fixed gram positive cells, we included the detergents EDT20, SDS, Triton X-100, Tween 20, and Cetrimide (all from Sigma) at various concentrations. With the exception of Triton X-100, there detergents had no influence on the hybridization. Inclusion of 1% Triton X-100 in the hybridization buffer resulted in intracellular substrate precipitation within fixed cells of B. subtilis and Lactococcus lactis (harvested during exponential growth), using probe Eub338. **Since even in an actively growing pure culture only some cells were stained, enzyme-labeled oligonucleotides cannot be currently used for specific single-cell identification of gram positive bacteria** (emphasis added). We encountered similar problems with cells of Saccharomyces cerevisiae, and again attempts to make the intracellular RNA accessible by enzymatic treatment (lyticase and β-glucoronidase; both from Sigma) of the cells or detergent addition failed." Amann et al. at page 3010, col. 1, lines 28-35

Taken as a whole, Applicants reiterate that Amann et al. had very limited success with the analysis of gram positive bacteria using enzyme-labeled probes and no success with any yeast. Moreover, Amann et al. specifically teach that the analysis of gram-positive bacteria and yeast with enzyme-linked probes are believed to be frustrated by a lack of penetration of the large enzyme-linked probe through the cell wall of these organisms. In view of their apparently disappointing results, Amann et al.

concluded: "**Since even in an actively growing pure culture only some cells were stained, enzyme-labeled oligonucleotides cannot be currently used for specific single-cell identification of gram positive bacteria.**"

However, most reveling is the Examiner's conclusory statement that: "... **there are no teachings in Amann which indicate that the enzyme linked probes cannot be applied to the detection of yeasts** (emphasis added)." As can be seen from the Abstract as well as the background section, it was well accepted at the time Amann et al. performed their work that enzyme-linked probe were not suitable for the analysis of either gram-positive bacteria or yeasts. Amann et al. described very limited success with detecting gram-positive bacteria using enzyme-linked probes and no success with yeast. Accordingly, quite the opposite of the Examiner's stated position is true. That is, there was no known ability to use enzyme-linked probes for that analysis of yeast at the time Amann et al. performed their work because there were believed to be too large to penetrate the cell wall and Amann et al. specifically **teach away** from the application of enzyme-linked probes to the analysis of yeast in general and to Saccharomyces cerevisiae in particular.

In addition to the foregoing, a Declaration from Dr. Henrik Stender has been submitted herewith. It is believed that the Declaration of Dr. Stender stands as further support of Applicants arguments in support of patentability and specifically rebuts the Examiner's conclusion that: "... **there are no teachings in Amann which indicate that the enzyme linked probes cannot be applied to the detection of yeasts** (emphasis added)."

In her rebuttal, the Examiner then continues by stating: "The specification provides no teachings as to critical steps that must be performed in order to allow for the detection of yeasts by in-situ hybridization using enzyme-labeled probes and the claims clearly do not recite any critical steps which distinguish the claims over the prior art in-situ hybridization methods of detecting organisms using enzyme-linked probes." It is respectfully submitted that this statement is irrelevant to the nature of the rejection. Because this rejection is made under 35 U.S.C. §103(a), and not 35 U.S.C. § 102, it is clear that the presently claimed subject matter is novel. Accordingly, there is no requirement that the specification or claims articulate, or even that there be, any such critical steps.

Non-obviousness requires only that the ordinary practitioner be without motivation or reasonable expectation of successfully accomplishing the claimed subject matter.

In view of the foregoing remarks, as well as the amendment set forth herein, it is respectfully submitted that the present rejection of claims under 35 U.S.C. §103(a) over Kosse in view of Stender (1998) should be withdrawn. Reconsideration is requested.

(iv) Rejection based upon Kosse in view of Stender (1998) and Parton (5,905,038)

At paragraph 4 of the present Office Action, the Examiner has rejected claims 47-49 and 80-85 under 35 U.S.C. §103(a) as being unpatentable over Kosse in view of Stender (1998) and further in view of Parton (US 5,905,038). Applicants respectfully traverse this rejection and stand ready to appeal this rejection directly to the Board of Patent Appeals & Interferences if again maintained.

This rejection is cumulative with the rejection articulated in paragraph 3 of the Office Action. Accordingly, for the reasons described above, it is believed that this rejection cannot properly stand as being dependent upon an improper combination of Kosse and Stender (1998).

In addition to the foregoing, Applicants add that the rejection articulated at paragraph 4 of the present Office Action does not state any specific motivation to combine the references and is therefore *prima facia* deficient. Moreover, it is believed that the statement of the rejection is hindsight based and self-fulfilling.

In view of the foregoing remarks, as well as the amendment set forth herein, it is respectfully submitted that the present rejection of claims under 35 U.S.C. §103(a) over Kosse in view of Stender (1998) and in further view of Parton should be withdrawn. Reconsideration is requested.

(v) Rejection based upon De Wachter in view of Kosse and Stender (1998)

At paragraph 5 of the present Office Action, the Examiner has rejected claims 1-8, 10-12, 16, 18-19, 21-26, 29, 32, 46, 60-62, 86 and 87 under 35 U.S.C. §103(a) as being unpatentable over De Wachter in view of Kosse and in further view of Stender (1998). Applicants respectfully traverse this rejection and stand ready to appeal this rejection directly to the Board of Patent Appeals & Interferences if again maintained.

This rejection is cumulative with the rejection articulated in paragraph 3 of the Office Action. Accordingly, for the reasons described above, it is believed that this rejection cannot properly stand as being dependent upon an improper combination of Kosse and Stender (1998).

Furthermore, in formulating this rejection, the Examiner has argued: "De Wachter teaches an isolated nucleic acid consisting of the sequence of 18S rRNA of *Dekkera/Brettanomyces bruxellensis*. The 18S rRNA of De Wachter comprises the sequence of SEQ ID NO: 1 (see nucleotides 1066-1052 of GenBank Accession No. X58052). The nucleic acid of De Wachter **is considered to have the property** of being suitable as a probe for the detection, identification or quantitation of *Dekkera/Brettanomyces bruxellensis*.(emphasis added)" (OA at page 7) From this final statement, it is clear that the Examiner has taken the conclusory position that the entire sequence disclosed by De Wachter can be used as a basis to construct probes for the determination of *Dekkera/Brettanomyces bruxellensis*. Accordingly, the Examiner's argument is completely undermined where statements in the art relied upon by the Examiner to reject Applicant's claims challenges this conclusory position.

Kosse specifically teaches that only the 3' end of the 18S rRNA is accessible to fluorescently labeled probes and that the other variable regions of 18S rRNA were not accessible to *in-situ* hybridization. (Abstract and page 478, col. 2, first full paragraph). Moreover, the Examiner has previously **admitted** that Kosse: "... teaches that prior to *in situ* hybridization, yeast cell walls **must be permeablized** (emphasis added) and that probes **must be selected to yeast 18 rRNA which are fully accessible to probes** (emphasis added) (see page 478)." (OA at page 3) Consequently, it is not reasonable to expect any nucleobase sequence that is homologous to the gene sequence described by De Wachter will be useful to produce an *in-situ* hybridization probe and in fact the possible sequence of De Wachter that can be used for constructing probes suitable for determining *Dekkera/Brettanomyces bruxellensis* is very limited in view of the express disclosure of Kosse. Moreover, the specification is quite clear that the probing nucleobase sequence is the specific sequence recognition portion of the probe and designed to hybridize to a target sequence within yeast (Specification at page 14, lines 15-17). Clearly if the target sequence is not accessible, the probing nucleobase sequence cannot hybridize to said target. Accordingly, since there is no evidence presented by

the Examiner that nucleotides 1066-1052 of GenBank Accession No. X58052 are fully accessible to probes, the cited references simply do not provide motivation or reasonable expectation of successfully using Seq. ID No. 1 to determine *Dekkera/Brettanomyces bruxellensis.*

In addition to the foregoing, it is respectfully submitted that the rejection articulated at paragraph 5 of the present Office Action does not state any specific motivation to combine the references and is therefore *prima facia* deficient. Moreover, it is believed that the statement of the rejection is hindsight based and self-fulfilling.

In view of the foregoing remarks, it is respectfully submitted that the present rejection of claims under 35 U.S.C. §103(a) over De Wachter in view of Kosse and Stender (1998) should be withdrawn. Reconsideration is requested.

(vi) Rejection based upon De Wachter in view of Kosse and Stender (1998) and further in view of Parton

At paragraph 6 of the present Office Action, the Examiner has rejected claims 47-49 and 80-85 under 35 U.S.C. §103(a) as being unpatentable over De Wachter in view of Kosse and Stender (1998) in further view of Parton (US 5,905,038). Applicants respectfully traverse this rejection and stand ready to appeal this rejection directly to the Board of Patent Appeals & Interferences if again maintained.

This rejection is cumulative with the rejection articulated in paragraph 3 of the Office Action. Accordingly, for the reasons described above, it is believed that this rejection cannot properly stand as being dependent upon an improper combination of Kosse and Stender (1998).

In addition to the foregoing, it is respectfully submitted that the rejection articulated at paragraph 6 of the present Office Action does not state any specific motivation to combine the references and is therefore *prima facia* deficient. Moreover, it is believed that the statement of the rejection is hindsight based and self-fulfilling.

In view of the foregoing remarks, as well as the amendment set forth herein, it is respectfully submitted that the present rejection of claims under 35 U.S.C. §103(a) over De Wachter in view of Kosse, Stender (1998) and Parton should be withdrawn. Reconsideration is requested.

V. SUMMARY

It is believed that this response addresses all rejections set forth in the present Office Action and the application is in ready condition for allowance. In consideration of the preceding amendments and remarks, Applicants hereby respectfully request reconsideration of all pending claims (as amended herein), the withdrawal of all rejections set forth in the present Office Action and issue of a Notice of Allowance by The Office.

VI. INTERVIEW

If the Examiner believes a telephonic or personal interview would advance the prosecution of the subject application, the Examiner is invited to contact attorney Gildea during business hours at the telephone or facsimile numbers listed below.

VII. FEES

Except for the fee due for consideration of the petition under 37 C.F.R. §1.136(a), the fee due for consideration of the petition under 37 C.F.R. § 1.144 and the fee due for submission of the Notice of Appeal, it is believed that no additional fees are believed due The Office for consideration of this paper. If however, The Office determines that any other fee is due, authorization is hereby granted to charge any required fee associated with the filing and consideration of this paper to Deposit Account 02-3240.

VIII. CORRESPONDENCE/CUSTOMER NUMBER

Please send all correspondence pertaining to this document to:

Brian D. Gildea, Esq.
Applied Biosystems
15 DeAngelo Drive
Bedford, MA 01730

Telephone: 781-280-2824
Fax: 781-280-2940

IF NOT ALREADY DONE, PLEASE MATCH THIS CASE WITH CUSTOMER NUMBER **23544**

23544
[Insert Customer Number Bar Code]

Respectfully submitted
on behalf of Applicants,

Brian D. Gildea, Esq.; Reg. No. 39,995